

Supplemental Material

Particulate Matter, DNA Methylation in Nitric Oxide Synthase, and Childhood Respiratory Disease

Carrie V. Breton¹, Muhammad T. Salam¹, Xinhui Wang¹, Hyang-Min Byun², Kimberly D. Siegmund¹ and Frank D. Gilliland¹

¹Department of Preventive Medicine, Keck School of Medicine, University of Southern California, 1540 Alcazar Street, CHP 236, Los Angeles, CA

² Exposure, Epidemiology & Risk Program, Harvard School of Public Health, Boston, MA

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Tables

Supplemental Material, Table S1. Primer sequences and reaction conditions for *NOS* genes

| | Primer ^a | Sequence | Annealing Temp | PCR size(bp) |
|-------|---------------------|---|----------------|--------------|
| NOS1 | PCR Forward | NOS1- F:AGGTTGGTAATGAAGATATTAGAGAAT AG | 56.7°C | 223 |
| | PCR Reverse | NOS1-R(biotin) : TCACCCACTCATAACTAATAACCC | | |
| | PSQ sequencing | NOS1-SP:TTTAGGGATA | | |
| NOS2A | PCR Forward | iNOS(23151743)-F: AAAAATAATTTTGGATGGTATGG | TDOWN53 | 177 |
| | PCR Reverse | iNOS(23151567)-R(biotin): AAACTATCTAAAACCTACCCAATCCC | | |
| | PSQ sequencing | iNOS(23151671)-SP:TTTATAATTTGTAG | | |
| NOS2A | PCR Forward | iNOS(23150425)-F: TTAGGGTTAGGTAAAGGTATTTGT | TDOWN53 | 212 |
| | PCR Reverse | iNOS(23150214)-R(biotin): CAATTCTATAAAACCACCTAATAATCTTA A | | |
| | PSQ sequencing | iNOS(23150425)-SP: TTAGGGTTAGGTAAAGGTATTTGT | | |
| NOS2A | PCR Forward | iNOS(23145018)-F: GGAAGGTAGGGAAAGGAGGGTAGTT | TNCTD | 243 |
| | PCR Reverse | iNOS(23144776)-R(biotin): AAAAATCCTACAAAACAACCTACACAACC | | |
| NOS3 | PSQ sequencing | iNOS(23144840)-SP: GAGGGGTTGGG | TDOWN53 | 187 |
| | PCR Forward | NOS3-F: GGATATTGGGTTTTATTAA | | |
| | PCR Reverse | NOS3-R(biotin): CAATAAAAAAAACTCTCCA | | |
| | PSQ sequencing | NOS3-SP: TGGGATAGGG | | |

^a Primers were designed using MethPrimer software, with parameters for selection including a product size of 100~300bp, 50~60oC for primer Tm, and a primer size of 20~30bp.

Supplemental Material, Table S2. Distribution of PM_{2.5} and PM₁₀ across 8 Southern California communities (N=940)

| Cumulative average | PM_{2.5} (µg/m³) | | | | | PM₁₀ (µg/m³) | | | | |
|---------------------------|--|---------------|------------|------------|------------|---|---------------|------------|------------|------------|
| | Mean | Median | IQR | Min | Max | Mean | Median | IQR | Min | Max |
| 7 day | 13.8 | 11.5 | 9.3 | 2.1 | 39.4 | 30.2 | 27.0 | 19.8 | 8.5 | 63.6 |
| 1 month | 13.5 | 12.2 | 7.1 | 5.2 | 26.9 | 29.4 | 30.2 | 11.4 | 15.5 | 54.6 |
| 6 month | 15.4 | 15.3 | 7 | 8.1 | 24.7 | 35.9 | 33.8 | 13.8 | 21.5 | 67.8 |
| 1 year | 16.7 | 15.8 | 4.8 | 9.7 | 23.7 | 38.3 | 33.2 | 14.6 | 28.2 | 57.7 |

Supplemental Material, Table S3. Spearman correlation between cumulative average air pollutant exposures in 940 selected CHS participants

| | 7 day PM _{2.5} | 1 month PM _{2.5} | 6 month PM _{2.5} | 1 year PM _{2.5} ^a | 7 day PM ₁₀ | 1 month PM ₁₀ | 6 month PM ₁₀ | 1 year PM ₁₀ ^a |
|---------------------------|----------------------------|------------------------------|------------------------------|--|---------------------------|-----------------------------|-----------------------------|---|
| 7 day PM _{2.5} | 1 | 0.70 | 0.56 | 0.56 | 0.80 | 0.63 | 0.52 | 0.47 |
| 1 month PM _{2.5} | | 1 | 0.80 | 0.73 | 0.56 | 0.81 | 0.71 | 0.68 |
| 6 month PM _{2.5} | | | 1 | 0.93 | 0.38 | 0.59 | 0.85 | 0.75 |
| 1 year PM _{2.5} | | | | 1 | 0.46 | 0.53 | 0.78 | 0.84 |
| 7 day PM ₁₀ | | | | | 1 | 0.77 | 0.52 | 0.49 |
| 1 month PM ₁₀ | | | | | | 1 | 0.75 | 0.63 |
| 6 month PM ₁₀ | | | | | | | 1 | 0.86 |
| 1 year PM ₁₀ | | | | | | | | 1 |

^a sample size is 843 for 1 year cumulative exposures

p <0.0001 for all correlations

Supplemental Material, Table S4. Spearman pairwise correlations for *NOS2A* CpG loci

| | Position 1 | Position 2 | Position 3 | Position 4 | Position 5 | Position 6 | Position 7 |
|------------|------------|------------|------------|------------|------------|------------|------------|
| Position 1 | 1 | 0.46* | 0.14* | 0.06 | -0.06 | -0.09* | -0.02 |
| Position 2 | | 1 | 0.06* | 0.05 | 0.03 | -0.04 | -0.02 |
| Position 3 | | | 1 | 0.12* | 0.07* | -0.03 | 0.02 |
| Position 4 | | | | 1 | 0.34* | 0.20* | 0.25* |
| Position 5 | | | | | 1 | 0.30* | 0.42* |
| Position 6 | | | | | | 1 | 0.35* |
| Position 7 | | | | | | | 1 |

*p<0.05

Supplemental Material, Table S5. Spearman pairwise correlations for *NOS1* and *NOS3* CpG loci

| | | <i>NOS1</i> | | | <i>NOS3</i> | |
|-------------|------------|-------------|------------|------------|-------------|------------|
| | | Position 1 | Position 2 | Position 3 | Position 1 | Position 2 |
| <i>NOS1</i> | Position 1 | 1 | 0.50* | 0.63* | -0.05 | -0.08* |
| | Position 2 | | 1 | 0.46* | -0.02 | -0.05 |
| | Position 3 | | | 1 | -0.08* | -0.10* |
| <i>NOS3</i> | Position 1 | | | | 1 | 0.41* |
| | Position 2 | | | | | 1 |

*p<0.05

Supplemental Material, Table S6. The difference in % DNA methylation in *NOS2A* non-CpG island per 5 µg/m³ change in cumulative PM_{2.5} exposure restricted to a non-asthmatics and non-wheezers, using beta regression^a

| Average PM _{2.5} exposure | Non-CpG island (promoter) | | | | | | Non CpG island (between exons 1 and 2) | | | CpG island ^c average of Position 4-7 | | |
|--|---|----------------|-----|---|---------------|-----|---|----------------|-----|--|------------------|-----|
| | Position 1 | | | Position 2 | | | Position 3 | | | | | |
| | Differenc e in % methylation ^b | 95%CI | N | Differen ce in % methylation ^b | 95%CI | N | Differenc e in % methylation ^b | 95%CI | N | Differen ce in % methylation ^b | 95%CI | N |
| 7 day | -0.26 | (-0.40, -0.12) | 689 | -0.01 | (-0.14, 0.13) | 686 | -0.38 | (-0.64, -0.12) | 704 | 0.09 | (0.01, 0.17) | 720 |
| 1 month | -0.44 | (-0.76, -0.12) | 689 | -0.15 | (-0.46, 0.16) | 686 | -0.31 | (-0.90, 0.28) | 704 | 0.18 | (-0.02, 0.37) | 720 |
| 6 month | -0.88 | (-1.32, -0.44) | 689 | -0.18 | (-0.57, 0.25) | 686 | -0.62 | (-1.39, 0.15) | 704 | 0.23 | (-0.03, 0.50) | 720 |
| 1 year | -1.27 | (-1.87, -0.67) | 612 | -0.51 | (-1.12, 0.11) | 609 | -0.39 | (-1.39, 0.61) | 626 | 0.40 | (0.02, 0.78) | 641 |

^a Beta regression coefficients were transformed to a linear scale to reflect a change in methylation in response to 5 µg/m³ increase relative to the mean pollutant level

^b All models were adjusted for age, sex, ethnicity, plate, month, town, and parental education

^c One subject with extremely high methylation value was considered an outlier and removed from analyses

Supplemental Material, Table S7. The difference in % DNA methylation in *NOS2A* and *NOS3* per 5 $\mu\text{g}/\text{m}^3$ increase in cumulative PM_{10} exposure, using beta regression^a

| Locus | Association | Average $\text{PM}_{2.5}$ exposure | | | |
|--------------------------------------|-------------------------|--|----------------|----------------|----------------|
| | | 7 day | 1 month | 6 month | 1 year |
| <i>NOS2A</i> Non-CpG island | Position 1 | Difference in % methylation ^b | -0.10 | -0.05 | -0.21 |
| | | 95% CI | (-0.18, -0.01) | (-0.18, 0.09) | (-0.38, -0.04) |
| | | N | 896 | 896 | 896 |
| | Position 2 | Difference in % methylation ^b | 0.06 | 0.15 | 0.07 |
| | | 95% CI | (-0.01, 0.14) | (0.03, 0.27) | (-0.08, 0.22) |
| | | N | 892 | 892 | 892 |
| | Position 3 | Difference in % methylation [†] | -0.11 | -0.09 | -0.47 |
| | | 95% CI | (-0.26, 0.05) | (-0.35, 0.17) | (-0.79, -0.15) |
| | | N | 916 | 916 | 916 |
| <i>NOS2A</i> CpG island ^c | Average of Position 4-7 | Difference in % methylation ^b | 0.01 | -0.01 | 0.04 |
| | | 95% CI | (-0.04, 0.05) | (-0.08, 0.07) | (-0.06, 0.13) |
| | | N | 939 | 939 | 939 |
| <i>NOS3</i> | Position 1 | Difference in % methylation ^b | 0.23 | 0.24 | 0.75 |
| | | 95% CI | (-0.06, 0.52) | (-0.25, 0.73) | (0.18, 1.32) |
| | | N | 914 | 914 | 914 |
| | Position 2 | Difference in % methylation ^b | 0.20 | 0.01 | 0.68 |
| | | 95% CI | (-0.04, 0.44) | (-0.42, 0.43) | (0.19, 1.17) |
| | | N | 818 | 818 | 818 |

| N | 914 | 914 | 914 | 818 |
|---|-----|-----|-----|-----|
|---|-----|-----|-----|-----|

^a Beta regression coefficients were transformed to a linear scale to reflect a change in methylation in response to 5 $\mu\text{g}/\text{m}^3$ increase relative to the mean pollutant level

^b All models were adjusted for age, sex, ethnicity, plate, month, town, and parental education, and asthma status

^c One subject with extremely high methylation value was considered an outlier and removed from analyses